UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/815,533	03/16/2001	Achille Arini	515-4218	9691

7590 08/27/2002

James V. Costigan, Esq.
HEDMAN & COSTIGAN, P.C.
Suite 2003
1185 Avenue of the Americas
New York, NY 10036-2646

EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 08/27/2002

10

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/815,533	ARINI ET AL.	
	Examin r	Art Unit	
	David J. Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the corresp ndence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 June 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 40-68* is/are pending in the application.

4a) Of the above claim(s) *56-68* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 40-55* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Application Status

Claims 1 and 40-68 are pending in the application.

Applicants' election with traverse of Group I, original claims 1-18, cancellation of claims 2-39 and addition of claims 40-68 in Paper No. 9, filed 06/03/02 is acknowledged.

Election/Restrictions

1. Applicants traverse the restriction requirement on the grounds that a single inventive concept (the use of alkanoic acids for the production of proteins) underlies at least Groups I-V. Applicants' argument is not found persuasive. While the inventions of Groups I-VII may be related by the use of alkanoic acids, the inventions are clearly distinct and require a separate search for the reasons provided as set forth in Paper No. 8. Applicants have not addressed the restriction requirement in regards to reasons why the inventions are not distinct or would not require a separate search. Therefore, the requirement is still deemed proper and is therefore made FINAL.

2. In view of applicants' amendments to incorporate the limitations of the claims of Groups II and III into the claims of Group I, claims 1 and 40-55 will be co-examined.

3. Claims 56-68 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Information Disclosure Statement

4. It is noted that an Information Disclosure Statement (Form PTO-1449) has been filed with the instant application as Paper No. 6. However, references CA and CB fail to comply with the requirements for an IDS as there is no publication date provided for either of the cited references. See 37 CFR 1.98 and MPEP § 609 regarding content of an IDS. Upon submission of an IDS in proper form, the examiner will consider the references and return Form PTO-1449 in a subsequent communication.

Sequence Compliance

5. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825; applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). Applicant is required to comply with the sequence rules by inserting the sequence identification numbers of all sequences recited within the specification at, for example, pages 13 and 21, and all other instances in the specification. See particularly 37 CFR 1.821(d).

Specification/Informalities

6. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The following title is suggested: "Production and Purification of Low Molecular Weight and High Molecular Weight Two-Chain Urokinase". See MPEP § 606.01.

Claim Objections

7. Claims 1 and 40 are objected to in the recitation of "an eukaryotic" in claim 1 and "a eukaryotic" in claim 40. It is suggested that applicants use the term "a eukaryotic" in both claims 1 and 40 to maintain consistency of the claims.

8. Claims 41 (claim 42 dependent therefrom), 44 (claim 45 dependent therefrom), 46 (claim 47 dependent therefrom) are objected to in the recitation of "chosen among". In the interest of clarity, it is suggested that applicants replace the term "chosen among" with "selected from the group consisting of".

9. Claims 43 and 44 are objected to because of the following informalities: the terms "a ion exchange" in claim 43, "further purify" in claim 43, "butyric acid sodium butyrate" in claim 44, are grammatically incorrect and should be replaced with, for example, "ion exchange" or "an ion exchange" in claim 43, "further purifying" in claim 43, and "butyric acid, sodium butyrate" in claim 44. Appropriate correction is required.

10. Claims 42 and 43 are objected to because of the recitation of "tc-UPA" in claims 42 and 43, "HMW" in claim 43, and "LMW" in claim 43. Abbreviations, unless otherwise obvious and/or commonly used in the art, should not be recited in the claims without at least once reciting the entire phrase for which the abbreviation is used. For example, in claim 42, the phrase for which the abbreviation is used can be stated as follows: "two-chain urinary type plasminogen activator (tc-uPA)" and subsequent claims need only recite "tc-uPA". Appropriate correction is required.

11. Applicant is advised that should claim 1 be found allowable, claim 40 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

12. Claims 1 and 40-55 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claims 1, 40 (claim 41 dependent therefrom), and 42 are indefinite in the recitation of "mature recombinant protein". One of skill in the art would recognize that the term "mature" in regards to a protein can be interpreted in a plurality of ways and would therefore not necessarily recognize the scope of recombinant proteins encompassed by the term "mature recombinant protein". It appears from the specification and the claims that the term is meant to be interpreted as a functional form of a recombinant protein that is expressed in a nonfunctional pre-cursor

form and the claims have been examined accordingly. It is suggested that applicants clarify the meaning of the term.

b. Claim 41 (claim 42 dependent therefrom) is indefinite in the recitation of "factors belonging to the cascade of the complement system". The term is indefinite as it is unclear as to applicants' intended scope of "factors". It is suggested that applicants clarify the meaning of the term.

c. Claim 42 is confusing in the recitation of "pre-proenzyme is pre-prourokinase *or prourokinase*" (italics added). The claim is confusing because prourokinase is not a pre-proenzyme. It is suggested that applicants replace "pre-proenzyme" in claim 42 with "protein precursor".

d. Claim 43 (claims 44-55 dependent therefrom) recites the limitation "the human pre-prourokinase" in lines 3 and 4. There is insufficient antecedent basis for this limitation in the claim.

e. Claim 43 (claims 44-55 dependent therefrom) is confusing as it is unclear as to whether steps d) and e) are part of the step c) ion exchange chromatography, or if steps d) and e) are to take place following and separately from the ion exchange chromatography step. The examiner has interpreted the claim as meaning that steps d) and e) are part of the step c) ion exchange chromatography. If the examiner's interpretation is correct, it is suggested that applicants incorporate steps d) and e) into the step c) ion exchange chromatography step. If the Examiner's interpretation of the claim is incorrect, Applicant should so state and clarify the record.

f. Claims 43 (claims 44, 46, 47, and 52 dependent therefrom), 45, 48-51, and 53-55 are indefinite in the recitation of "comprised between". It is unclear from the claims and the specification as to whether the pH values, concentrations, temperatures, or times are between those values specifically recited in the claims, or encompass other pH values, concentrations, temperatures, or times that have not been recited in the claims. As written, one of skill in the art

would not recognize the scope of applicants' intended pH values, concentrations, temperatures, or times. It is suggested that applicants clarify the meaning of the claims.

g. The term "low-salt solution" in step d3) of claim 54 and step e3) of claim 55 is a relative term and it is unclear from the claims as to applicants' intended concentration that is considered "low-salt". It is suggested that applicants clearly identify the intended concentration of salt that is considered "low-salt".

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

13. Claims 1, 40, and 41 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1, 40, and 41 are directed to a method of producing a genus of mature recombinant proteins into the culture medium of a eukaryotic cell transfected with a genus of cloned precursor cDNA sequences or cloned cDNA sequences of the precursor proteins of claim 41, by incubating the cells in a culture comprising alkanoic acids or a derivative or salt thereof. The specification teaches a method of producing only a single representative species of such mature recombinant proteins, i.e., two-chain urokinase (tc-uPA) using a cDNA encoding pre-pro-urokinase. Moreover, the specification fails to describe any other representative species of precursor proteins by any identifying characteristics or properties other than the functionality of being a mature recombinant protein, a precursor protein, or a precursor protein as recited in claim 41. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear,

concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

14. Claims 1 and 40-55 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-urokinase (pre-pro-UK) comprising the steps of: a) incubating said cell line in a cell culture medium comprising sodium butyrate for a time of at least 24 hours; b) recovering the resulting supernatant; c) purifying the LMW and HMW tc-uPA by SP-Sepharose ion exchange chromatography as follows: i) eluting low molecular weight (LMW) tc-uPA from SP-Sepharose by addition of a buffer with a pH between 5.5 and 6.5, comprising a monovalent cation concentration between 200 and 300 mM and optionally further purifying LMW tc-uPA by benzamidine chromatography; and e) eluting high molecular weight (HMW) tc-uPA from SP-Sepharose by addition of a buffer solution with a pH value between 6 and 7.5 comprising a monovalent cation concentration of at least 400 mM and optionally further purifying HMW tc-uPA by benzamidine chromatography, does not reasonably provide enablement for either of a method for the production of *any* mature recombinant protein into the culture medium of a eukaryotic cell transfected with *any* cloned precursor cDNA sequence or *any* of the precursors of claim 41, by incubating the cells in a culture comprising *any* alkanoic acid or a derivative or salt thereof or a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-UK comprising the steps of: a) incubating said cell line in a cell culture medium comprising *any* alkanoic acid or a derivative or salt thereof for a time of at least 24 hours; b) recovering the resulting supernatant; c) performing ion exchange chromatography using *any* ion exchange matrix on the supernatant as follows: i) eluting LMW tc-uPA by addition of a buffer with a pH between 5.5 and 6.5, comprising *any* monovalent ion (anion or cation) concentration between 200 and 300 mM and optionally further purifying LMW tc-uPA by benzamidine chromatography; and e) releasing the HMW tc-uPA by addition of a buffer solution with a pH value between 6 and 7.5 comprising *any* monovalent ion (anion or cation) concentration of at least 400 mM and optionally further purifying HMW tc-uPA by benzamidine chromatography. The specification

does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 1 and 40-55 are so broad as to encompass a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-UK using in the method *any* alkanoic acid or a derivative or salt thereof, *any* ion exchange matrix on the supernatant, an elution buffer for elution of LMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration between 200 and 300 mM, and an elution buffer for elution of HMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration of at least 400 mM as described above. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of alkanoic acids or a derivatives or salts thereof, ion exchange matrices, and elution buffers comprising monovalent ions. In the instant case, the disclosure is limited to a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-UK comprising the steps of: a) incubating said cell line in a cell culture medium comprising sodium butyrate for a time of at least 24 hours; b) recovering the resulting supernatant; c) purifying the LMW and HMW tc-uPA by SP-Sepharose ion exchange chromatography as follows: i) eluting LMW tc-uPA from SP-Sepharose by addition of a buffer with a pH between 5.5 and 6.5, comprising a monovalent cation concentration between 200 and 300 mM and optionally further purifying LMW tc-uPA by benzamidine chromatography; and e) eluting HMW tc-uPA

from SP-Sepharose by addition of a buffer solution with a pH value between 6 and 7.5 comprising a monovalent cation concentration of at least 400 mM and optionally further purifying HMW tc-uPA by benzamidine chromatography.

The specification does not support the broad scope of the claims which encompass a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-UK using in the method *any* alkanoic acid or a derivative or salt thereof, *any* ion exchange matrix on the supernatant, an elution buffer for elution of LMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration between 200 and 300 mM, and an elution buffer for elution of HMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration of at least 400 mM as described above because the specification does not establish a rational and predictable scheme for culturing a host cell in the presence of *any* alkanoic acid or a derivative or salt thereof with an expectation of obtaining the desired recombinant protein as applicants have provided guidance by demonstrating only a single working example using sodium butyrate. The specification does not provide guidance for practicing the claimed method using *any* alkanoic acid or a derivative or salt thereof.

Also, the specification does not establish a rational and predictable scheme for using *any* ion exchange matrix with an expectation of obtaining the desired protein using an elution buffer for elution of LMW tc-uPA comprising *any* monovalent ion (anionic or cationic) concentration between 200 and 300 mM, and an elution buffer for elution of HMW tc-uPA comprising *any* monovalent ion (anionic or cationic) concentration of at least 400 mM the buffer. One of skill in the art would recognize that the ability of an ion exchange matrix to purify a protein is dependent upon the ion concentration of the elution buffer and *any* ion exchange matrix will not necessarily be useful for purification of LMW and HMW using elution buffers with *any* monovalent ion concentration as recited in claim 43 parts d) and e). Therefore, without further guidance, the specification is only enabling for purification of LMW and HMW tc-uPA using SP-Sepharose (as disclosed in the specification at page 19) using the buffers as recited in claim 43 parts d) and e).

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method for the production of tc-uPA from a eukaryotic cell line transfected with an expression vector comprising a nucleic acid encoding pre-pro-UK using in the method *any* alkanoic acid or a derivative or salt thereof, *any* ion exchange matrix on the supernatant, an elution buffer for elution of LMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration between 200 and 300 mM, and an elution buffer for elution of HMW tc-uPA comprising *any* monovalent ion (anion or cation) concentration of at least 400 mM as described above. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

15. Claims 1 and 40-42 are rejected under 35 U.S.C. 102(b) as being anticipated by Okabayashi et al. (Cell Struct Funct 14:579-586, 1989; hereafter referred to as "Okabayashi"). Claims 1 and 40 are drawn to a method for the production of an active recombinant protein in the culture medium of a eukaryotic cell line transfected with an expression vector for the expression of a cDNA encoding a precursor of the active protein by incubating the cell line in a medium containing alkanoic acids or salts or derivatives thereof for at least 24 hours. Claim 41 further limits the protein precursor of claim 40 and claim 42 further limits the pre-proenzyme and the mature recombinant protein of claim 41.

Okabayashi teaches a method for the expression of recombinant human urokinase using Chinese hamster ovary (CHO) cells cultured in a medium comprising sodium n-butyrate (page 579, abstract). Okabayashi teaches the CHO cells were transfected with an expression vector comprising a cDNA encoding human pre-prourokinase (page 580). Okabayashi teaches urokinase activity, measured following 24 hour incubation in culture medium comprising sodium n-butyrate, revealed significant increases in urokinase activity relative to untransfected CHO cells (page 582, Table 1). Because pre-prourokinase and prourokinase (sc-uPA) are known in the art to be catalytically inactive (see also page 1, lines 24 and 25 of the instant specification), the urokinase present in the culture medium comprising sodium n-butyrate as taught by Okabayashi would have inherently been active urokinase, i.e., tc-uPA. Since the Office does not have the facilities for examining and comparing applicants' method of producing active recombinant protein with the method of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the method of the prior art is not the same as the claimed method). See *In re* Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re* Fitzgerald et al., 205 USPQ 594. This anticipates claims 1 and 40-42 as written.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. Claims 43-49 and 53-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okabayashi in view of Nobuhara et al. (J Biochem 90:225-232, 1981; hereafter referred to as "Nobuhara"), and Miwa et al. (Chem Pharm Bull 29:463-471, 1981; hereafter referred to as "Miwa").

Claim 43 is drawn to a process for the production of mature recombinant HMW and LMW tc-uPA from a

eukaryotic cell transfected with a cDNA encoding human pre-prourokinase by culturing said cell in the presence of alkanoic acids, derivatives, or salts thereof, recovering the produced supernatant and performing ion exchange chromatography and optionally benzamidine chromatography to purify LMW tc-uPA from HMW tc-uPA as encompassed by the claims. Claims 44 and 45 limit the alkanoic acid and concentration thereof of claim 43. Claims 46 and 46 limit the cell of claim 43. Claims 48 and 49 limit the temperature of cell incubation of claim 43.

Okabayashi discloses the teachings as described above. Okabayashi does not teach the limitations of separating the HMW and LMW forms of tc-uPA by ion exchange chromatography and optionally further purifying either of HMW or LMW tc-uPA by benzamidine chromatography and optionally further purifying either of HMW or LMW tc-uPA by gel filtration chromatography.

The therapeutic use of urokinase as a thrombolytic agent was well known to one of ordinary skill in the art at the time of the invention. As such, methods of purifying the HMW and LMW forms of tc-uPA using ion exchange, benzamidine, and gel filtration chromatography in various combinations using buffers comprising various salt concentrations at varying pH values were all well known in the art at the time of the invention as shown by the representative references of Nobuhara and Miwa. It is noted that additional references describing the purification of HMW and LMW forms of tc-uPA were identified by the examiner. However, these references were not cited as the references of Nobuhara and Miwa are representative of the prior art.

Nobuhara discloses purification of HMW and LMW forms of tc-uPA using sequential column chromatography as follows: Amberlite cation exchange chromatography using a buffer comprising 10 mM sodium phosphate, pH 8.0 with 0.5 M sodium chloride followed by Sephadex G-100 gel filtration chromatography using 0.1 M phosphate buffer at pH 8.0 containing 0.3 M sodium chloride followed by benzamidine chromatography using 0.1 M acetate buffer at pH 4.0 containing 0.4 M sodium chloride, followed by Sephadex G-100 gel filtration chromatography (page 226 left column).

Miwa discloses a method of purifying HMW and LMW forms of tc-uPA using sequential column chromatography as follows: Sephadex G-100 gel filtration chromatography using a 20 mM sodium glycine

buffer comprising 0.3 M sodium chloride at pH 8.6, Sephadex G-75 column chromatography using a 20 mM sodium glycine buffer comprising 0.3 M sodium chloride at pH 8.6, and SP-Sephadex ion exchange chromatography using a linear gradient of 0 to 0.4 M sodium chloride to elute the HMW and LMW forms of tc-uPA (pages 464-466). Miwa teaches that using the SP-Sephadex C-50 cation exchanger allowed for the purification of various subforms of HMW and LMW tc-uPA, which is not achieved with all cation exchangers (page 466). Miwa teaches that the SP-Sephadex cation exchange step provided purified fractions with the highest specific activity and allowed for the highest level of specific activity ever obtained at the time of publication (pages 466 and 467). Miwa teaches a motivation for purifying the HMW and LMW forms of tc-uPA at page 467 by disclosing that HMW tc-uPA has been shown to be a pharmacologically advantageous form of urokinase as it has been shown to exhibit a higher thrombolytic capacity in vivo relative to LMW tc-uPA.

As demonstrated by the cited references representative of the state of the art at the time of the invention, methods of purifying the HMW and LMW forms of tc-uPA were well known to one of ordinary skill in the art. While the examiner recognizes that neither Nobuhara nor Miwa teaches a purification scheme or buffers used for elution of the HMW and LMW forms of tc-uPA as specifically recited in the claims, one of ordinary skill in the art would have recognized that the buffers used for a particular chromatography would depend on such factors as the specific chromatography matrix, the manufacturer's recommendations, and the relative stability of the desired protein. Therefore, it is clear from the cited representative prior art references that methods of isolating HMW and LMW tc-uPA using various combinations of chromatography matrices in differing orders with different buffers were well known to one of ordinary skill in the art. Thus, based on the state on the prior art, it is well within the ability of an ordinarily skilled artisan to purify HMW from LMW forms of tc-uPA using ion exchange chromatography and optionally benzamidine chromatography and gel filtration chromatography using the buffers as recited in the claims.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Okabayashi, Nobuhara, and Miwa to practice the method of Okabayashi for producing tc-uPA and

purifying the HMW form of tc-uPA from the LMW form of tc-uPA using ion exchange chromatography and optionally benzamidine chromatography and gel filtration chromatography. One would have been motivated to purify the HMW form of tc-uPA from the LMW form of tc-uPA because of the relatively higher thrombolytic activity of HMW tc-uPA. One of ordinary skill in the art would have a reasonable expectation of success for practicing the method of Okabayashi for producing tc-uPA and purifying the HMW tc-uPA from the LMW tc-uPA using ion exchange chromatography and optionally benzamidine chromatography and gel filtration chromatography because of the teachings of Okabayashi, Nobuhara, and Miwa, and the state of the prior art and the ability of one of ordinary skill in the art as discussed above. Therefore, claims 43-49 and 53-55, drawn to a the methods of producing HMW and LMW tc-uPA as described above would have been obvious to one of ordinary skill in the art.

17. Claims 50-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okabayashi in view of Nobuhara, and Miwa as applied to claims 43-49 and 53-55 above, and further in view of Hu et al. (Sheng Wu Gong Cheng Xue Bao 16:387-391, hereafter referred to as "Hu"). Claims 50 and 51 limit the time of cell incubation of claim 43 and claim 52 limits the culture medium for the cell of claim 43.

Okabayashi, Nobuhara, and Miwa disclose the teachings as described above. The references of Okabayashi, Nobuhara, and Miwa do not combine to teach the limitations of claims 50-52.

Hu teaches the production of urokinase using CHO cells cultured in serum free medium up to 91 days (abstract).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Okabayashi, Nobuhara, Miwa, and Hu to practice the method of Okabayashi for producing tc-uPA by culturing the cells according to Hu and purifying the HMW form of tc-uPA from the LMW form of tc-uPA by ion exchange chromatography and optionally benzamidine chromatography and gel filtration chromatography. One would have been motivated to culture the cells by the method of Hu in order to produce tc-uPA in large quantities as required for commercial therapeutic use. One would have a reasonable expectation of success for practicing the method of Okabayashi for producing tc-uPA by culturing cells according to Hu and purifying the HMW tc-uPA from the LMW tc-uPA because of the

teachings of Okabayashi, Nobuhara, Miwa, and Hu, and the state of the prior art and the ability of one of ordinary skill in the art as discussed above. Therefore, claims 50-52, drawn to a the methods of producing HMW and LMW tc-uPA as described above would have been obvious to one of ordinary skill in the art.

Conclusion

18. No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Friday from 7:30 am to 2:00 pm and from 3:30 pm to 5:30 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Group is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600